Seasonal regulation of petal number

McKim, Sarah M.; Routier-Kierzkowska, Anne-Lise; Monniaux, Marie; Kierzkowski, Daniel; Pieper, Bjorn; Smith, Richard S.

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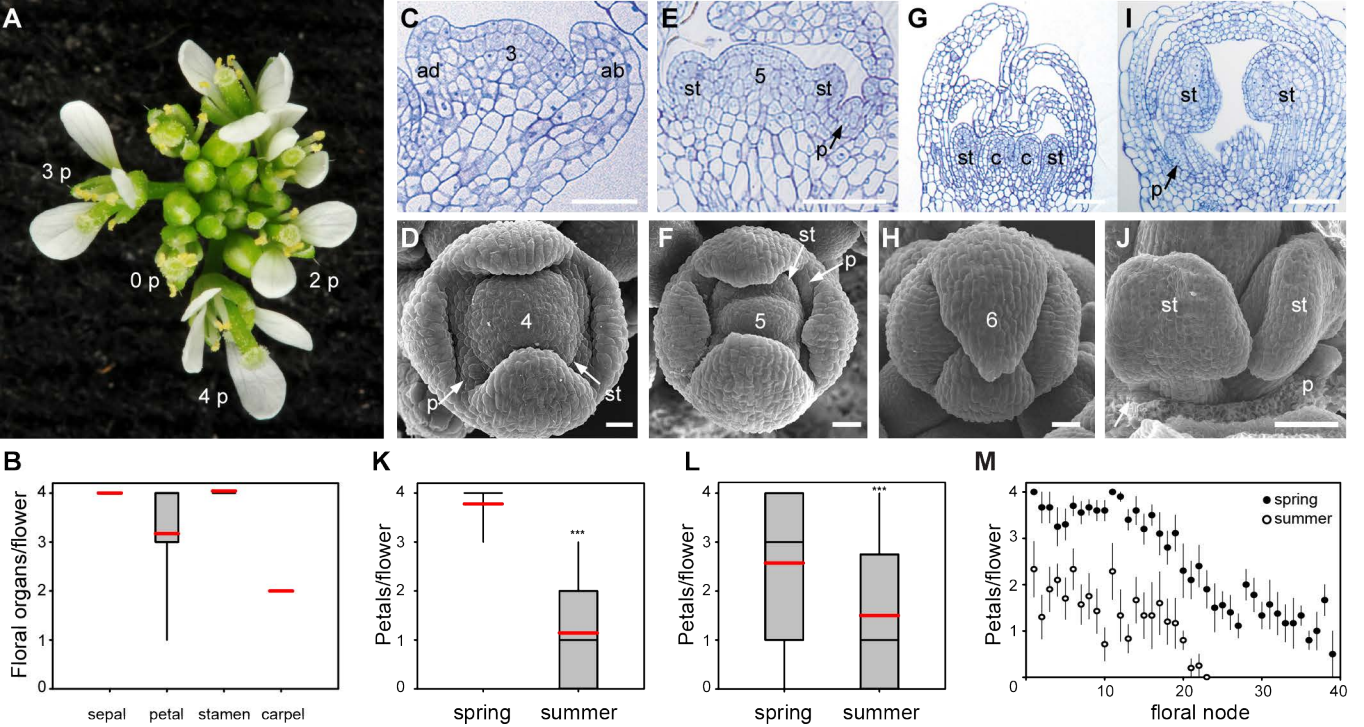

Figure 1. Petal number varies according to season and age in *C. hirsuta*. (A) Petal number varies between zero and four in the flowers of a single inflorescence in *C. hirsuta*. (B) Boxplot of floral organ counts per flower in *C. hirsuta*. (C) Longitudinal section through a stage 3 floral bud shows growth of the abaxial (ab) sepal is advanced compared to the adaxial (ad) sepal. (D) Scanning electron micrograph (SEM) of a stage 4 floral bud shows outgrowth of all four sepals and early petal and stamen primordia. (E) Longitudinal section and (F) SEM of stage 5 floral buds show sepals growing over the floral meristem, prominent stamen primordia and small petal primordia. (G) Longitudinal section and (H) SEM of stage 6 flowers show sepals enclosing the bud with their tips folding within the bud. Section shows developing stamen and carpel primordia but no petal primordia. (I) Longitudinal section and (J) SEM of older floral buds show that petal primordia are either present or absent (arrow, J) in whorl two. (K) Boxplot of petals per flower in spring and summer flowering populations of C. hirsuta, n=419 flowers. (L) Boxplot of petals per flower in the *C. hirsuta* Ox accession grown in late spring and summer field conditions, n = 468 flowers. (M) Average petal number per flower at consecutive floral nodes in *C. hirsuta* Ox plants grown in summer (white circles) and late spring (black circles) field conditions, n = 10 plants in each condition, error bars show standard error of the mean. The first floral node is labelled as 1. Pair-wise comparisons by Mann-Whitney U test. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Boxplots show 25th to 75th percentiles, whiskers extend down to 10th and up to 90th percentiles, black line shows median, red line shows mean. Numbers in (C-H) indicate floral stage; st, stamen; p, petal; c, carpel. Scale bars: 20 μm (C-J).

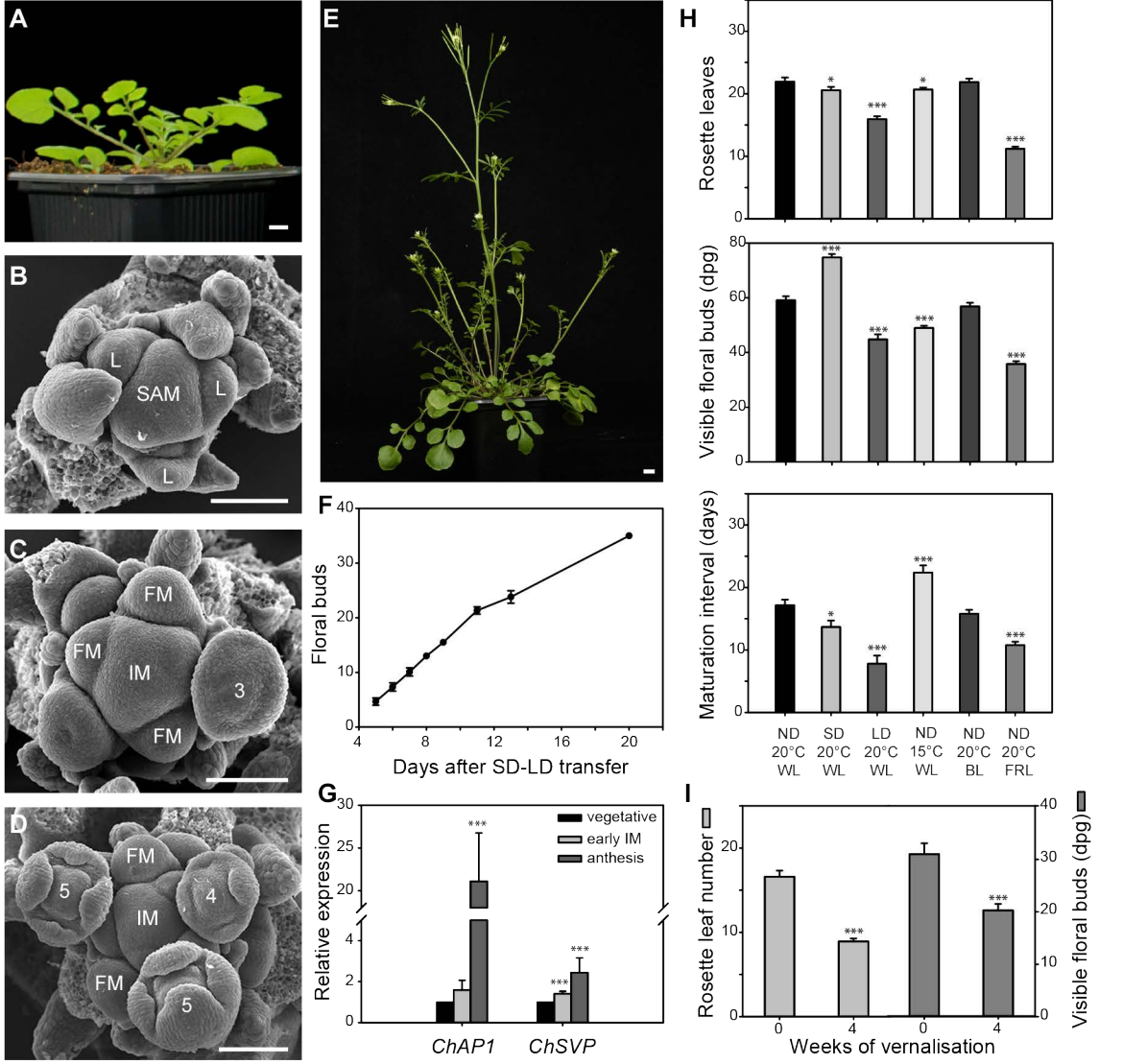

Figure 2. *C. hirsuta* flowering is accelerated by long days, vernalisation, cool ambient temperature and simulated shade. (A) *C. hirsuta* vegetative rosette. (B-D) SEMs of *C. hirsuta* plants following SD to LD transfer: (B) shoot apical meristem (SAM) initiating leaf primordia (L) four days after transfer, (C) inflorescence meristem (IM) initiating floral meristem primordia (FM) five days after transfer with the oldest primordium at stage 3, (D) IM initiating FM six days after transfer with the oldest primordium at stage 5. (E) *C. hirsuta* flowering raceme. (F) Line graph shows cumulative floral bud production starting five days after SD to LD transfer. (G) Relative expression levels of ChAP1 and ChSVP mRNA in vegetative SAM, early IM (5-10 floral buds), and IM at anthesis, shown as fold-change of vegetative levels. Expression is compared pair-wise to vegetative samples. (H) Flowering time, measured as number of rosette leaves and number of days post-germination (dpg) when the first floral buds are visible, and floral maturation interval, measured as the number of days between visible floral buds and anthesis, were compared between different photoperiods, ambient temperature, and light quality. Abbreviations: ND, neutral days (12:12); SD, short days (8:16); LD, long days (16:8); WL, white light; BL, blue light; FRL, far-red light. (I) Flowering time, measured as number of rosette leaves (left y-axis) and visible floral buds (right y-axis), in response to vernalisation at ~4°C. Pair-wise comparisons by Mann-Whitney U test. Significance levels: *** p<0.001, ** p<0.01, * p<0.05. N = 26-28 (ND, 20°C, WL), n = 7-26 (LD, 20°C, WL), n = 26-29 (SD, 20°C, WL), n = 12-31 (ND, 15°C, WL), n = 29-31 (ND, 20°C, FRL), n = 22-31 (ND, 20°C, BL), n = 12 (vernalization). Error bars show standard error of the mean. Scale bars: 1 cm (a,e), 100 µm (b-d).

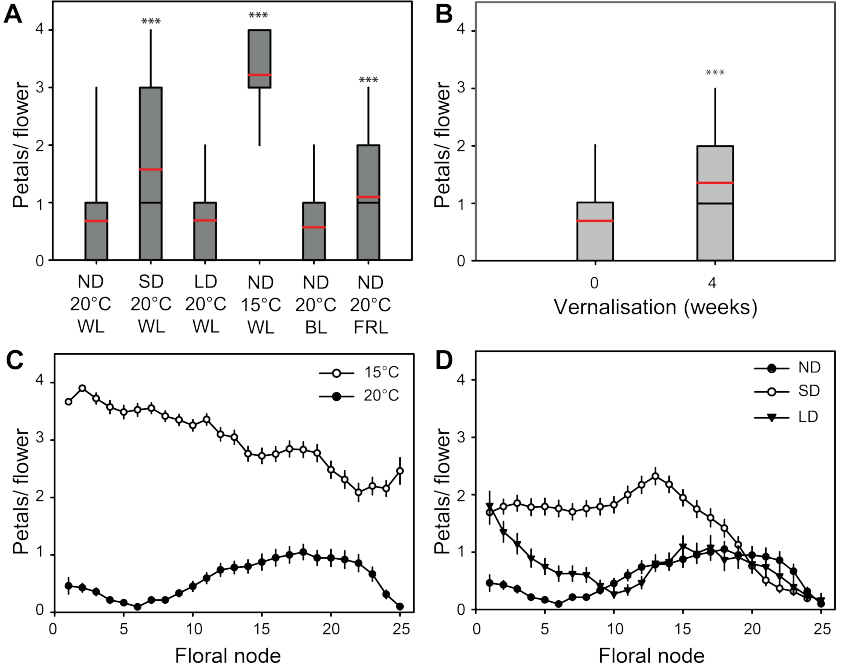

Figure 3. Petal number in *C. hirsuta* responds to photoperiod, ambient temperature, light quality and vernalisation. (A-B) Boxplots compare petal number per flower between different photoperiods, ambient temperature, and light quality (A), and in response to vernalisation at ~4°C (B). (C-D) Average petal number per flower over successive floral nodes in response to ambient temperature (C) and to photoperiod (D), the first floral node is labelled as 1, error bars show standard error of the mean. Petals/flower are scored in the first 25 floral nodes in (A, B). Pair-wise comparisons by Mann-Whitney U test. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Abbreviations: ND, neutral days (12:12); SD, short days (8:16); LD, long days (16:8); WL, white light; BL, blue light; FRL, far-red light. N = 26-28 (ND, 20°C, WL), n = 7-26 (LD, 20°C, WL), n = 26-29 (SD, 20°C, WL), n = 12-31 (ND, 15°C, WL), n = 29-31 (ND, 20°C, FRL), n = 22-31 (ND, 20°C, BL), n = 12 (vernalization). Boxplots show 25th to 75th percentiles, whiskers extend down to 10th and up to 90th percentiles, black line shows median, red line shows mean.

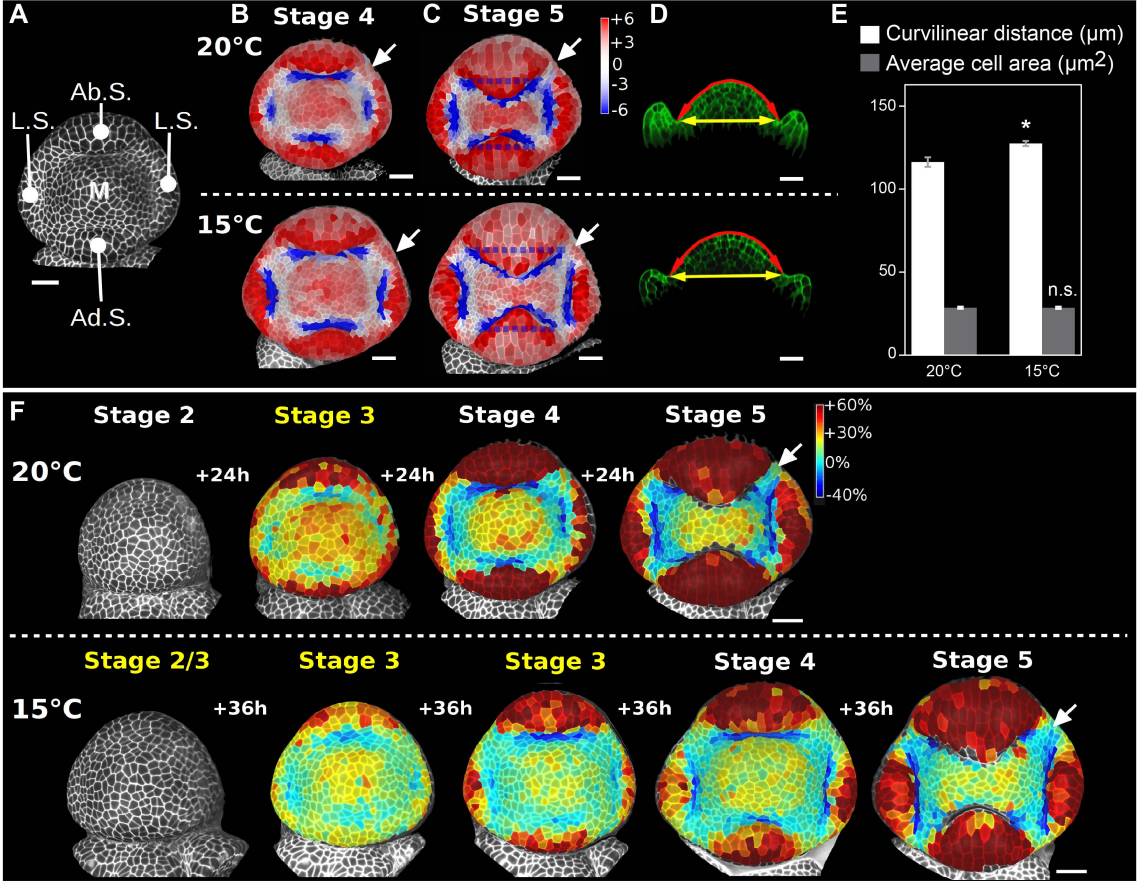

Figure 4. Ambient temperature affects the size of inter-sepal regions in *C. hirsuta* floral buds. (A) Early floral development in *C. hirsuta* was staged by the size and shape of sepals (lateral sepals: L.S., abaxial sepal: Ab.S., adaxial sepal: Ad.S.) according to Table S2. All subsequent floral buds are oriented this way. (B-C) Local curvature computed with MorphoGraphX software in stage 4 (B) and stage 5 (C) floral buds of *C. hirsuta* at 20°C (upper) and 15°C (lower). Heatmaps indicate tissue curvature in 10-2 µm-1, where flat is white, negative curvature is blue, and positive curvature is red. Arrows indicate flat regions between sepals. Dashed blue lines approximate the sepal-meristem boundaries when these are obstructed by sepal growth in (C). (D) Optical cross-sections of stage 4 floral buds of *C. hirsuta* at 20°C (upper) and 15°C (lower). Two measures of floral meristem size are indicated: the direct distance (yellow arrow) and the curvilinear distance (red arrow) between lateral sepal boundaries. (E) Barplot of curvilinear distance across the floral meristem (white) and epidermal cell area in the floral meristem (grey) of stage 4 floral buds of *C. hirsuta* at 15°C and 20°C. Curvilinear distance is significantly different between temperatures ($p < 0.05$, Student's t-test). (F) Areal growth computed with MorphoGraphX software in time-lapse series of *C. hirsuta* floral buds at 20°C (upper) and 15°C (lower). Arrows indicate regions of slower growth between sepals. Heatmap indicates cell areal growth in percentage over intervals of 24 h (20°C, upper) or 36 h (15°C, lower). Stage 3 is marked in yellow to highlight the considerable delay in development through this stage at 15°C. Note the 15°C series is composed of two different time-lapse series (see Fig. S11), and the heatmap is saturated for high growth in the sepals in order to discriminate between growth in the floral meristem and inter-sepal regions. Scale bars: 20 µm.

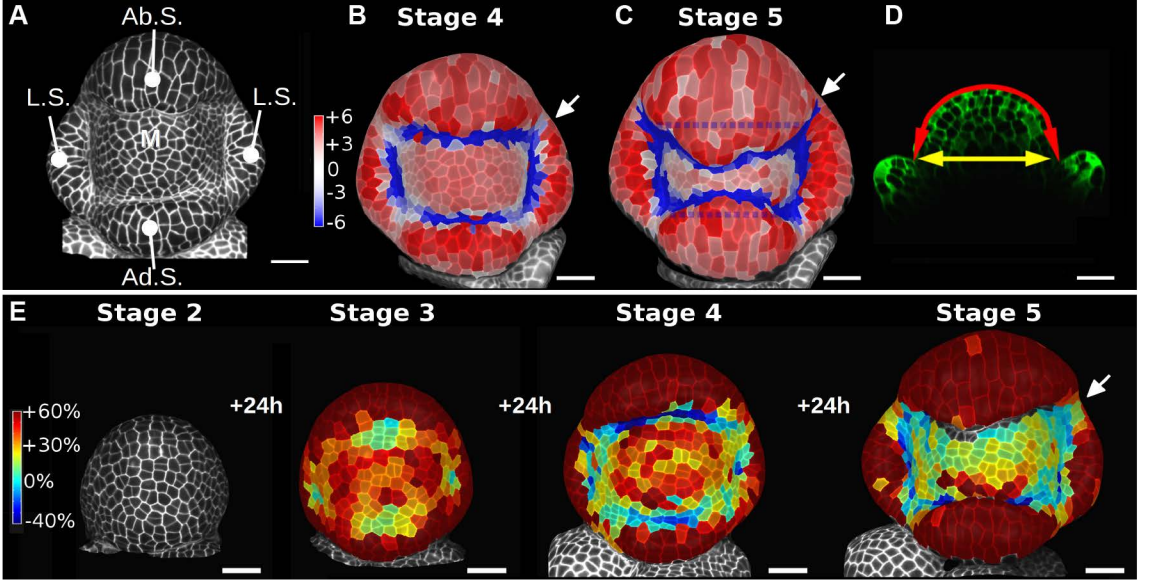

Figure 5. Geometry of the inter-sepal regions in *A. thaliana* floral buds differs from *C. hirsuta*. (A) Early floral development in *A. thaliana* was staged by the size and shape of sepals (lateral sepals: L.S., abaxial sepal: Ab.S., adaxial sepal: Ad.S.) according to (Smyth et.al., 1990). All subsequent floral buds are oriented this way. (B-C) Local curvature computed with MorphoGraphX software in stage 4 (B) and stage 5 (C) floral buds of *A. thaliana* at 20°C. Heatmap indicates tissue curvature in 10-2 µm-1, where flat is white, negative is blue, and positive is red. Arrows indicate inter-sepal regions. Dashed blue lines approximate the sepal-meristem boundaries when these are obstructed by sepal growth in (C). (D) Optical cross-section of stage 4 floral bud showing two measures of floral meristem size: the direct distance (yellow arrow) and the curvilinear distance (red arrow) between lateral sepal boundaries. (E) Areal growth computed with MorphoGraphX software in time-lapse series of *A. thaliana* floral buds at 20°C. Arrow indicates inter-sepal region with slower growth. Heatmap indicates cell areal growth in percentage over intervals of 24 h. Note the heatmap is saturated for high growth in the sepals in order to discriminate between growth in the floral meristem and inter-sepal regions. Scale bars: 20 µm.